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Synthesis and Characterization of 1,2,4-triazole and their diazotized compound as Bio-active agents

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ABSTRACT

The 1,2,4-triazole ring is a component of a wide range of biologically active compounds. Prompted by the biological properties and chemotherapeutic activity of 1,2,4-triazoles, 5-phenyl-4-amino-3-mercapto 1,2,4-triazole and its diazotized compound were synthesized. 1,2,4-triazole synthesized was characterized by UV-Visible, NMR & IR study. The absorption spectra of the investigated triazoles were recorded in different organic solvents of different polarity at room temperature. The absorption spectra showed bathochromic shift when solvent was changed from aprotic to protic. The absorption spectra of diazotized compound also showed bathochromic shift when coupled with β-napthol, phenol and N, N-dimethylaniline. This is due to presence of auxochromic group in conjugation. Cytotoxicity and antiviral assay of the 1,2,4-triazole and their diazotized compounds were performed in vivo and vitro.

Keywords: 1,2,4-triazol, diazotized compound, 5-phenyl-4-amino-3-mercapto 1,2,4- triazole, antiviral activity.

INTRODUCTION

Synthesis of 1,2,4-triazole is a very important because of component of wide range of biologically activity. 1,2,4-triazole is a chemotherapeutically important in various medicines. During the last few decades, a considerable attention has been devoted to the synthesis of 1,2,4-trizole and its derivatives. The synthesis of 1,2,4-triazole ring system was first of all reported by Bladin[1].

Nitro-1,2,4-triazoles have a specific application as explosive materials[2]. Triazoles and in particular 1,2,4-triazole nucleus have been incorporated into a wide variety of therapeutically interesting drug including anti-inflammatory, central nervous system (CNS) stimulants, sedatives, anti-anxiety and antimicrobial agents[3-4]. Their antifungl activity is also documented[5-6]. Candida albicans and Cryptococcus neoformans are two of the most common opportunistic fungi responsible for fungal infections in human beings. Rabodinirina *et al.*[7] and Hood *et al.*[8], have reported that the frequency of deeply invasive caniddiasis has increased ten fold during the past decades. Moreover, many infections due to candida species are actually refractory to

antifungal therapy. While these new classes of compounds are frequently used in treatment of fungal infections, resistance to these drugs is rising, which clearly indicates an urgent need for new antifungal agents.

Xavier *et al*.[9] reported the synthesis and antifungal activity of some mono and disubstituted amino mercapto triazoles. Many of these derivates exhibit high activity against candida albicans and candida tropicals.

Reports have also been found regarding fused 1,2,4-triazole *(Figure 1)* which express antifungal[10], bactericidal[11], anxiolytic[12-13], anticonvulsant[14] or herbicidal[15] activities or can act as antidepressants[16].

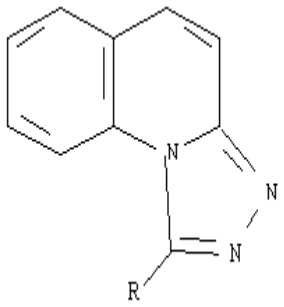

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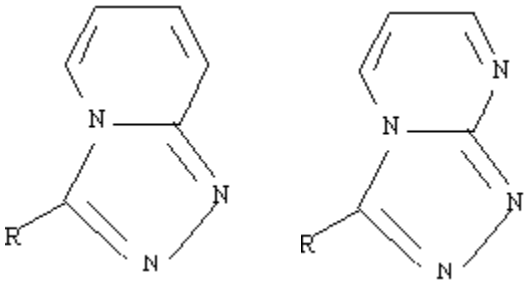
Figure 1. Five different types of fused 1,2,4-triazoles.



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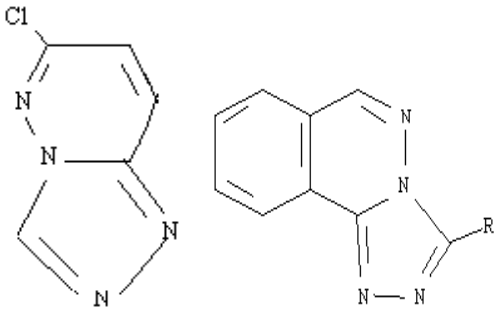

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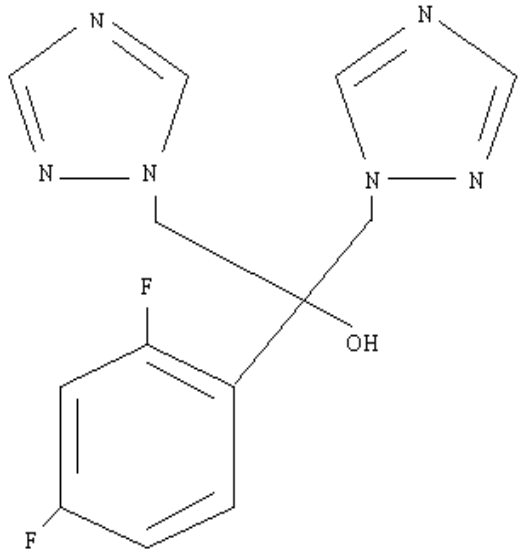
Two important examples of 1,2,4-triazoles which act as antifungal and antiviral agent; antifungal agent fluconazole and the antiviral agent nucleoside ribavirin *(Figure 2)*.



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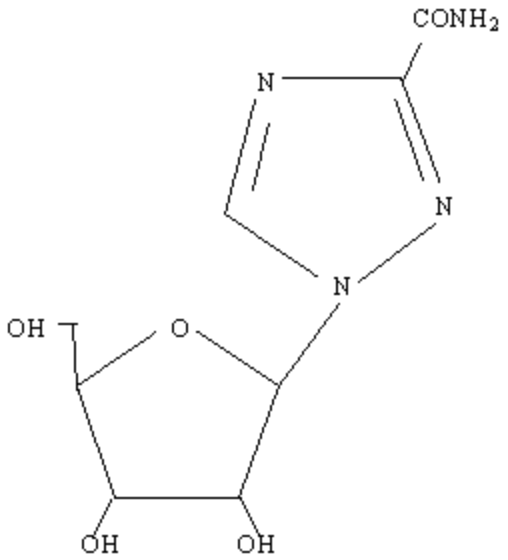

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Figure 2. Antifungal agent fluconazole (6) and the antiviral nucleoside ribavirin (7).

In a recent study the use of bis-hetero cycles containing 1,2,4-triazole and 1,2,4-triazine ring system as antihaemostatic drugs has been reported by Kamble and Sudha[17].

Holla et al.[18] reported the synthesis of a series of 7-arylidene-6-[2,4-dichlorophenyl]-3-aryloxymethyl/anilinomethyl-1,2,4-triazole [3,4-b]-1,3,4-thiadiazines by the condensation of 3-aryl-1-[2,4- dicholoro-5-fluorophenyl] -2-bromo-propen-1-one and 4-amino-5- merc-apto-3-aryloxymethyl/aniline methyl-1,2,4-triazoles. The newly synthesized compounds were characterized by elemental analysis, IR, ^{1}HNMR and mass spectral data and were tested for their antimicrobial activities against Escherichia coli, Staphylococcus aureus, Psuedomonas arruginosa. Some of the newly synthesized compounds were also screened for their anticancer activity. Some of them showed in-vitro anticancer activity.

Triazole compounds have also attracted attention having been used as highly efficient and wide spectrum fungicide and plant growth regulators[19]. The triazole compounds containing, 3-dioxolane have the remarkable prevention of the control over plant diseases. Propiconazol and difenoconazole are two important representatives[20].

According to the concept of bioisosterism[21], the seven new triazole compounds containing 1,3-dioxolane were synthesized by Jian et al.[22]. Their structures were identified by means of elemental analysis, IR, ^{1}HNMR and mass spectra. The results from the primary biological test have been reported that all the compounds have some activities of fungicide and plant growth regulators.

In the last few decades, a considerable attention have also been devoted to the synthesis of 1,2,4- triazole derivatives and 4,5-dihydro-1H-1,2,4-triazol-5-ones possessing such diverse biological activities as analgestic[23], antibacterial[24-29], fungicidal[30-31], anti-inflammatory[32-33] antihypertensive[34-35], herbicidal[36-37], coccidiostatic[38], antiviral[39], antagonistic[40], controgestalional[41] and anti-tumoral[42-44]. Moreover, several compounds involving 1,2,4-triazole moiety and having diverse pharmacological and antitumorial activities were reported[45-51].

The antitumor activity of 3-alkyl-4-phenylethylidenamino and 3-alkyl-4-)-3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazole-5-ones has been reported by Dmirbas[52].

MATERIALS AND METHODS

In the preparation of starting materials, the reagents were distilled and thoroughly dried by common methods.

Benzene (b. pt. 80°C) from Merck chemicals and n-hexane (b. pt. 69°C) from Merck chemicals were dried over sodium wire

after refluxing for about 8 hours. The final drying was checked by addition of few crystals of benzophenone Merck chemicals, which results in the blue colorations, if the solvent is dried. Finally, the solvents were distilled and used. Acetonitrile (b. pt. 82°C) from Fluka chemicals were refluxed over phosphorous pentoxide for about 2-3 hours and finally distilled. Methanol (b. pt. 65°C) Fluka chemicals and ethanol (b. pt. 78°C) Merck chemicals was refluxed over iodine and magnesium turnings, distilled and stored over molecular sieve (3A). DMF from Acros organics were used after keeping over anhydrous $CaSO_4$ for two nights, dried and stored in desiccators.

The melting points of the compounds were determined on an Visual Melting range apparatus MR VIS, Lab India Pvt. Ltd.

Infrared spectra of the compounds have been recorded in KBr in the range 4000-200 cm^{-1} with Shimadzu IR spectrophotometer. ^{1}H-NMR spectra were recorded on Jeol 300 MHz at observation frequency 300.4 Hz (Obset 130.00 KHz for $CDCl_3$ and PW_1 5.00 us) using TMS as internal standard. UV absorption spectra of the compounds have been recorded in different solvents on the radiation 550-200 nm with SL-159 ELW-UV spectrophotometer.

SYNTHESIS OF 5-PHENYL-4-AMINO-3-MERCAPTO-1, 2, 4-TRIAZOLE

This synthesis involves four steps, starting with the preparation of ethyl benzoate (8) *(Figure 3)*

In the first step, the compound (8) was synthesized by reported literature method[55].

In the second step, synthesis of aromatic acid hydrazide (9) from an equimolar mixture of ethyl benzoate (8) (29 ml, 0.2 mol) and hydrazine hydrate (10 ml, 0.2 mol) in presence of ethyl alcohol was refluxed for 6 hours. The reaction mixture was cooled and then excess ethanol was distilled off. After cooling the reacting mixture, a colorless solid separated out which was filtered, dried and recrystallized from ethanol.

In the third step, synthesis of 2-phenyl-5-mercapto-1,3,4-oxadiazoles (10) from a solution of carboxylic acid hydrazide (9) (18.84 ml, 0.14 mol) KOH (7.8 gm, 0.14 mol), CS_2 (14 ml, 0.14 mol) and ethanol (70 ml.0.14 mol) was refluxed for 20-22 hours. After monitoring the reaction, excess ethanol was distilled off and the residual pasty mass was ice cooled and subsequently acidified with dil. HCl. A solid separate out which was filtered washed repeatedly with excess double distilled cold water and dried in air. Further, the filterate on acidification with concentrated HCl (from Fluka chemicals) resulted from ethanol. The completion of reaction was checked by TLS (25% EtOAC/n-hexane).

Lastly, in the fourth step, oxadiazole (0.1mol) and hydrazine hydrate (21.9 ml,0.25 mol) were taken in presence of water (10ml)/ the reaction mixture was refluxed for three hours till a homogeneous mixture was obtained. The completion of the reaction was checked by TLC (25%, 30%EtOH/n-heaxane) and was allowed to cool at room temperature. Subsequently, the reaction mixture was poured into acidified double distilled cold water. The solid separated out. It was filtered and purified by re-crystallization with dil. Ethyl alcohol. The product 5-phenyl-4-amino-3-mercapto-1,2,4-triazole[53] (11) thus obtained was characterized through spectral studies.

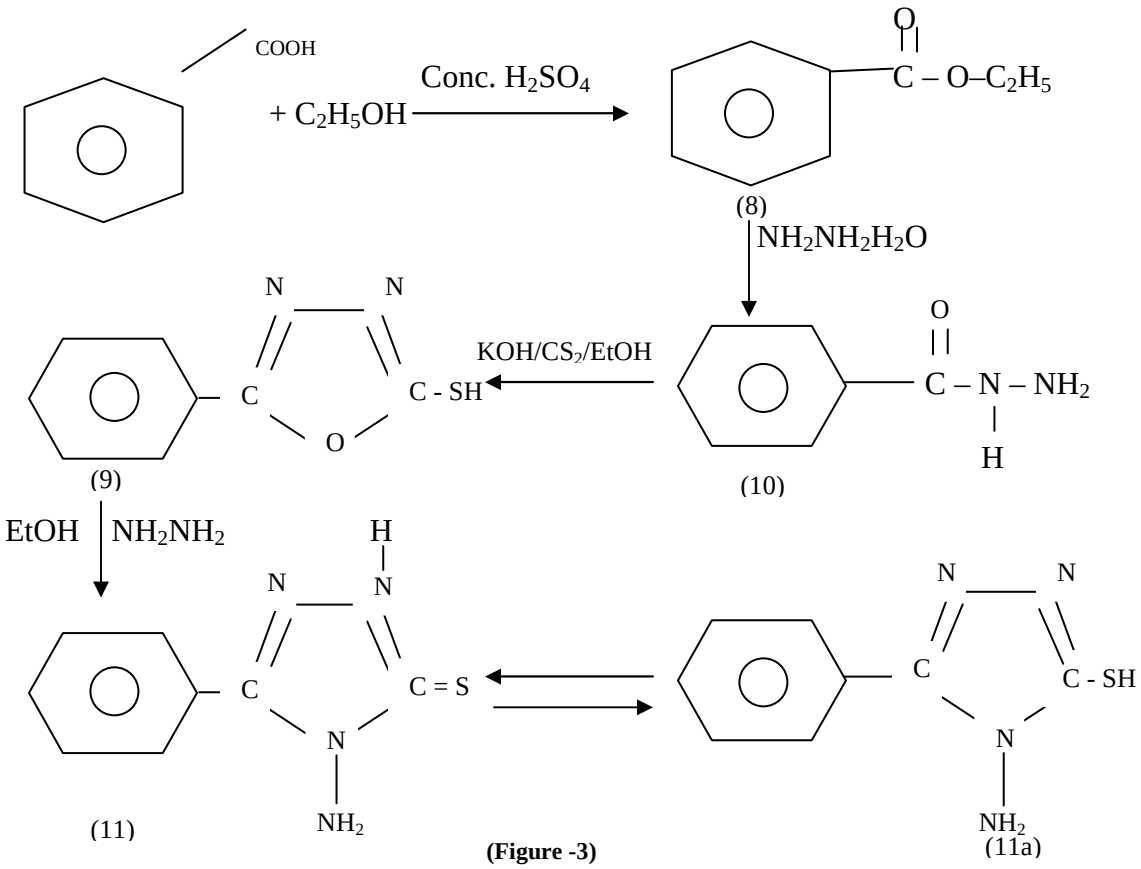
Yield 5.72gm, m. pt. 200ºC (lit. 202ºC); UV-VIS (EtOH) λ_{max} 241nm; IR V_{max} (cm^{-1}): 3328, 3310 (NH_2, Str.), 2350 (-CN), 2000-1750 (combination and overtone region), 1610 (C=N), 1600, 1560 (Ar), 1475 (NH, C=S), 1070 (C=S), 740, 710 (monosubstituted

benzene) ^{1}HNMR (DMSO-d_6) : δ 5.75 (2H, s, NH_2), 7.57-7.50 (5H, m, Ar-H), 13.7 (1H, s, NH).

DIAZOTISATION REACTION OF 5-PHENYL-4-AMINO-3-MERCAPTO-1,2,4-TRIAZOLE

A cold alcoholic triazole solution (11) was treated with dilute HCl then cold aqueous solution of $NaNO_2$ was added with continuous stirring drop by drop keeping the temperature maintained below 5°C. The diazonium salt obtained was added to the cold solution of phenol[56]. Phenol was coupled under alkaline condition mostly in the 4- position (12) *(Figure 4).*

M. Pt.. 260ºC (decompose)
IR V_{max} (cm^{-1}) : 2900 (phenolic-OH), 2550 (-SH), 2300(CN) 1640 (-N=N)1600, 1590 (C=C), -1200, 1370 (-C-O- Str.), 1515 (CN), 840 (disubstituted benzene); ^{1}HMR (DMSO,d_6) : δ 6.6 (1h,s,-SH), 8.4-7.9 (9H, m, Ar-H), 9.6 (1H, s, OH)
(ii) A cold alcoholic triazole solution (11) was treated with HCI then cold aqueous solution of $NaNO_2$ was added with continuous stirring drop by drop keeping the temperature maintained below 5°C. The diazonium salt thus obtained was added to the cold solution of β-naphthol. β-naphthol was coupled under alkaline condition gave the dye (13) *(Figure 5).*



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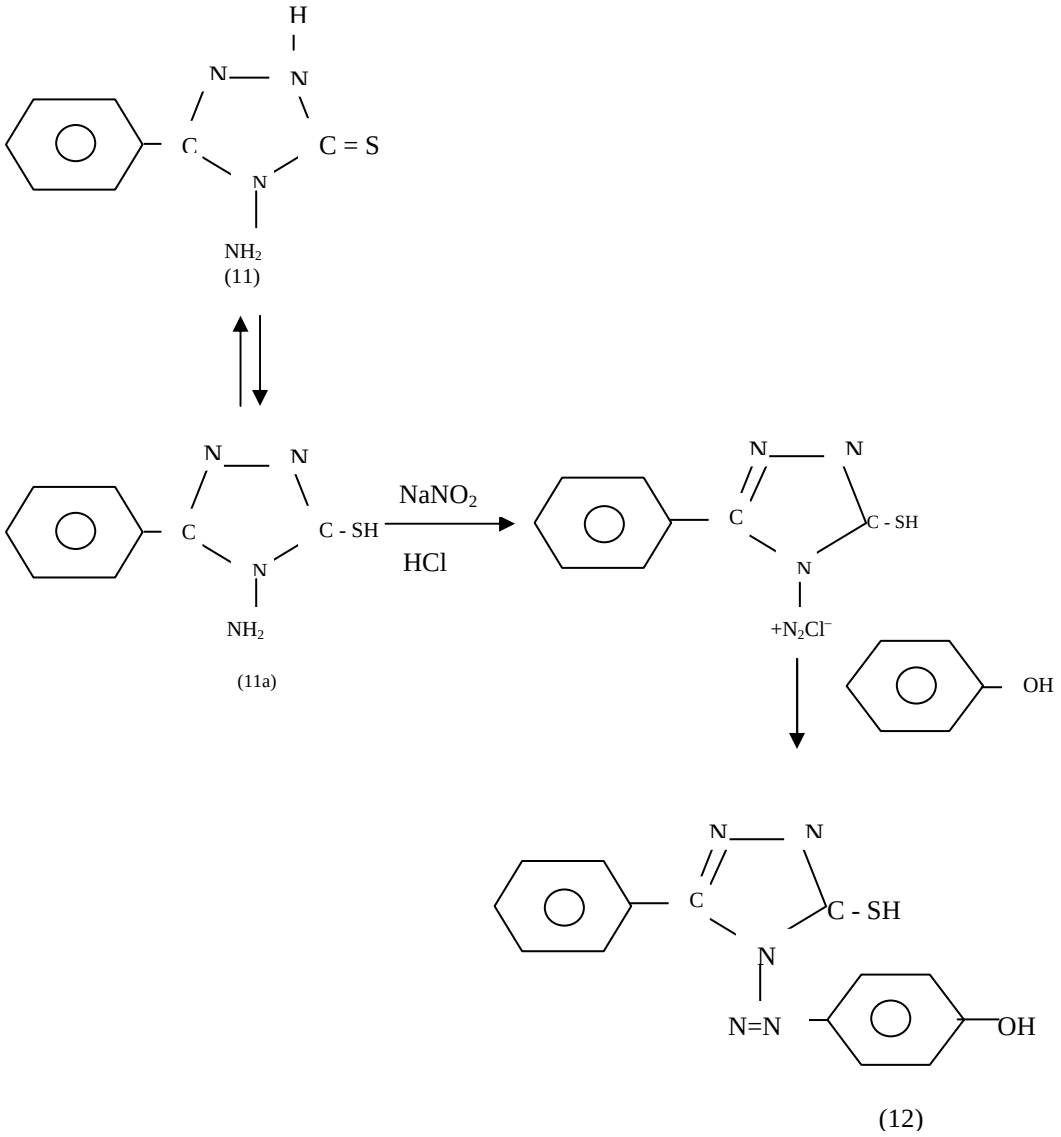


(Figure 4)

M. Pt. 212°C (decompose)
IR V_{max} (cm^{-1}) : 3040 (Ar-H); 2570(-SH), 2310 (-CN), 1575 (-N=N-), 1350, -1060 (-OH bending); ^{1}HNMR (DMSO, d6) : δ 6.6 (1H, s, SH), 7.29-7.20 (11 H, m, Ar-H).

(iii) A cold alcoholic triazole solution (11) was treated with dilute HCl then cold aqueous solution of $NaNO_2$ was added with continuous stirring drop by drop keeping the temperature maintained below 5°C. The diazonium salt

thus obtained was added to the cold solution of N, N-dimethyl aniline (14) *(Figure 6).*

UV-VISIBLE STUDIES OF SYNTHESIZED TRIAZOLE AND THEIR DYES IN THE DIFFERENT SOLVENT

The synthesis of triazole and their dye in the different solvent was done by dissolving them in the respective dry solvent i.e. ethanol, acetonitrile and dimethyl formamide. First of all, a stock solution was prepared of 10^{-3} conc. Mol/5 ml was done in different solvent. Same procedure was applied for the synthesis of different triazole and diazotized compound. The different solutions were subjected to UV studies for determination of absorption and λ_{max} respectively.

m. pt. 220°C (decompose)
IR V_{max} (cm^{-1}) : 3010 (Ar-H), 2310 (-CN), 1620 (-N=N-), 1200 (-SH), 960 (sub. Comp. – CH def)
^{1}HNMR (DMSO, d6): δ 2.47 (6H, s, $-CH_3$), 6.2 (1H, s, SH), 7.8-6.9 (9 H, m, Ar-H).

SYNTHESIS OF TRIAZOLE

(A) 5-phenyl-4-amino-3-mercapto-1,2,4-triazole.

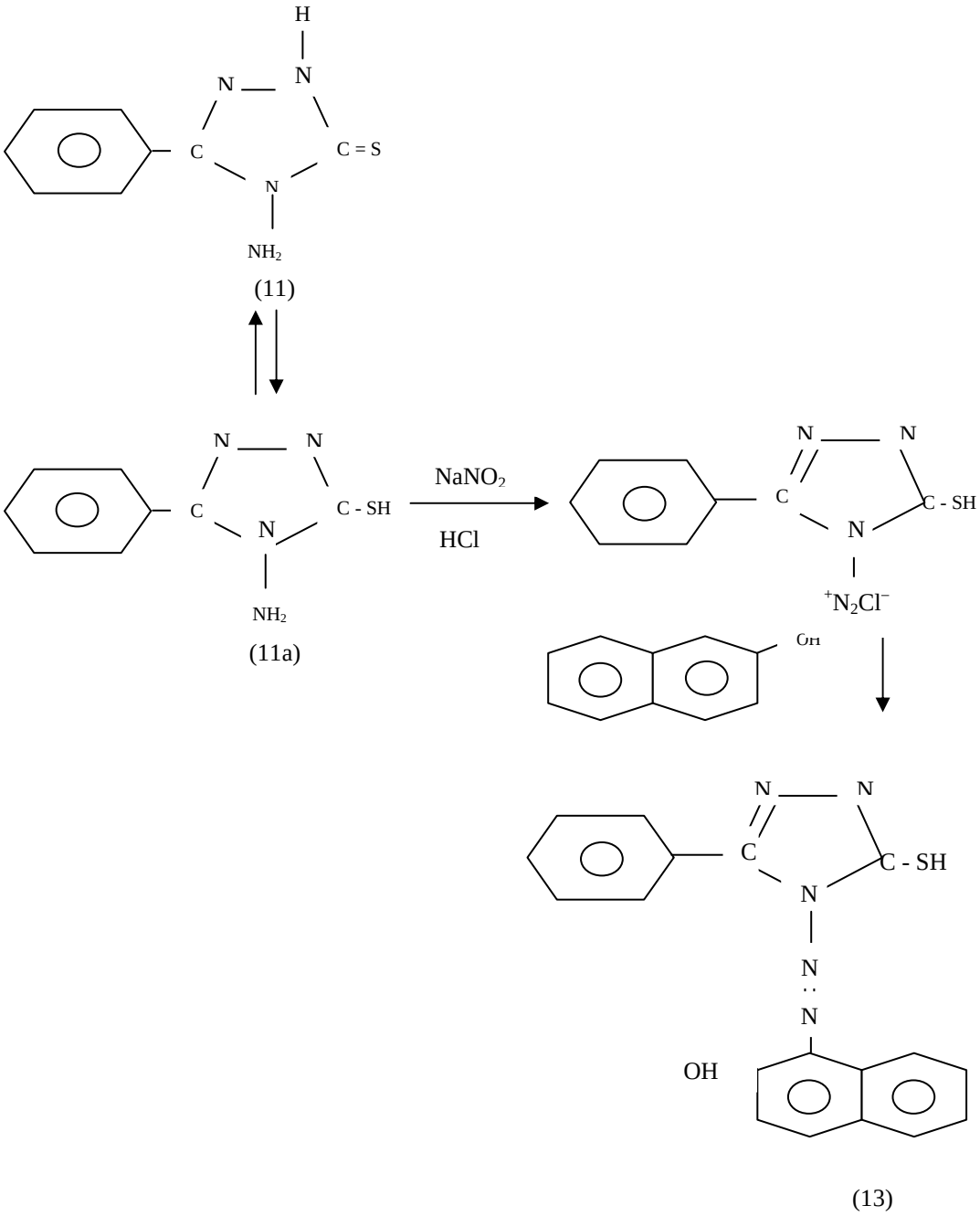
The synthesis of compound (11) was carried out by the reported literature method[51]. The m. pt. agrees with those reported in the literature[53]. Spectroscopic data of these compounds confirm the structure proposed and are listed in the experimental section. The structure of (11) has been confirmed on the basis of IR and ^{1}HNMR spectral studies.

IR Spectra: The IR spectra of compound (11) strong and sharp two band absorption at 3328, 3310 cm^{-1} indicative of the primary amine (-NH str.). The absorption at 2000-1700 cm^{-1} is characteristic overtone or combination band pattern indicative of monosubstituted aromatic group. Literature survey confirms the assignment of these band[57]. The absorption at 1610 cm^{-1} indicate the presence of C=N group. The characteristic absorption bands for C=C str. and NH-C=S appear at 1600-1560 cm^{-1} and 1475 cm^{-1} respectively. IR spectra confirm the presence of a strong band in the region of ~1070 cm^{-1} corresponds to the thione (-C=S) from[58]. The substitution of phenyl ring at position 5 confirm the appearance of absorption at 740, 710 cm^{-1} for the monosubstituted benzene *(Figure 7).*

^{1}H-NMR Spectra: the ^{1}H-NMR spectra of the compound were recorded in $CDCi_3$ + DMSO d_6 solution using TMS as internal reference. The one broad signals observed for NH protons at δ13.7 ppm indicates the greater stability of thione tautomeric form (**Fig 1).** The five proton multiplet at δ7.57-7.00 ppm due to the phenol attached to triazole ring. The signals for NH_2 protons appears as singlet at δ 5.75 ppm.

Diazotisation and Coupling Reactions of Synthesis Triazoles

Diazotisation of heterocyclic compound have been reported in the literature[54]. However not many reactions pertaining to diazotization of triazoles have been found in the literature survey. It was therefore worthwhile to explore the reactivity of synthesized triazole (11) towards Diazotisation reaction followed by coupling with compounds containing active hydrogen. The diazotization was carried out by the usual procedure with sodium nitrite and concentrate HCl. Further, coupling reactions were explored with β-naphthol, phenol, N, N-dimethylaniline


H
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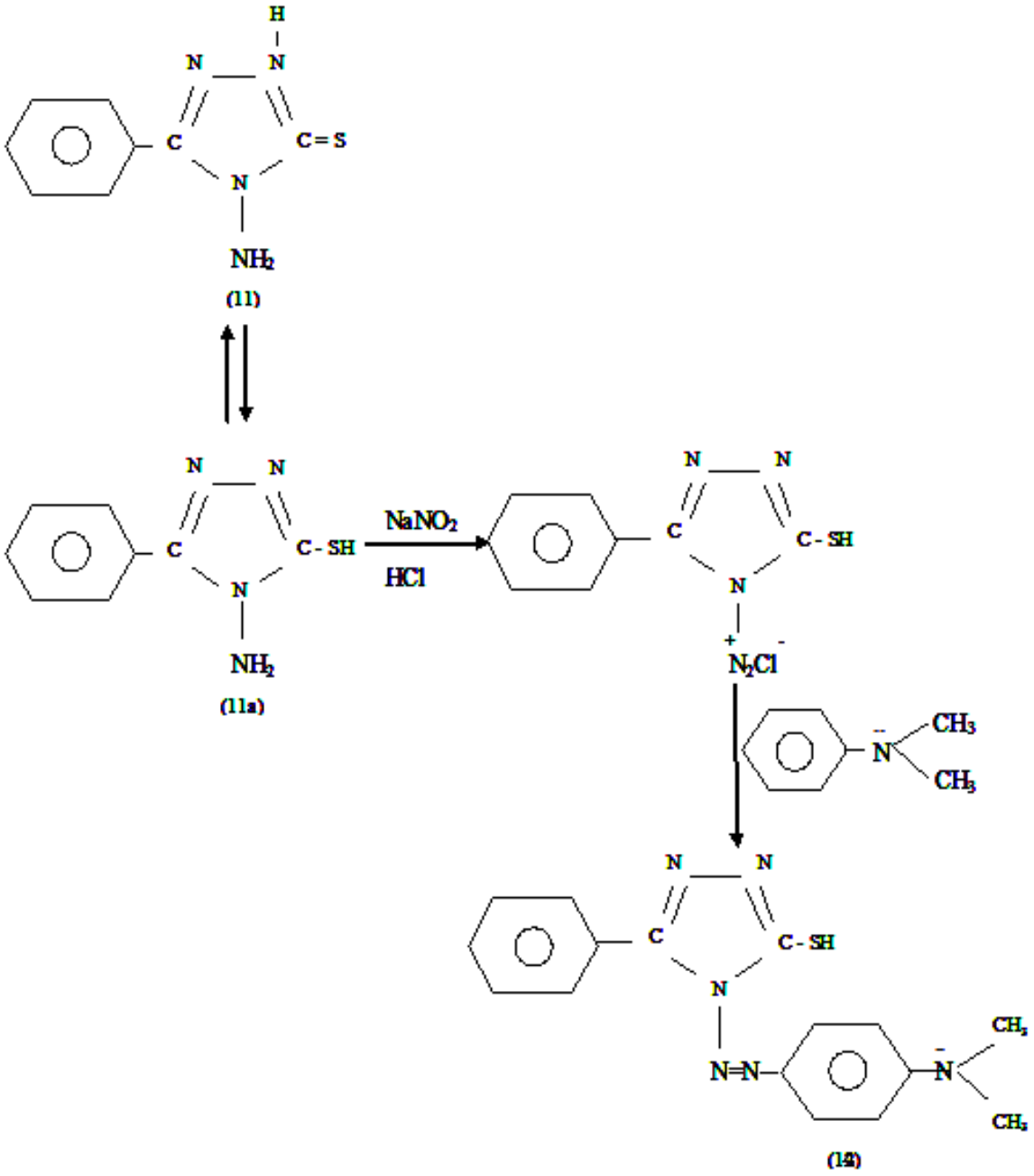
(Figure 5)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(12)

(Figure 6)

IR Spectra: in the IR spectra of compounds (12), (13) and (14), absence of absorption in the region 3500-3300 cm^{-1} indicating for the tertiary group (Fig. 4, 5 and 6) on comparing the IR Spectra of (12), (13) and (14) with that of (11), the absence of **"-NH str."** Band in the compound (12), (13) and clearly indicate the formation of diazotized coupled compound. In the IR spectra of compound (12) absorption broad band at 2900 cm^{-1} for the characteristics –OH str. in phenolic group is observed *(Fig. 8)*. In such a case of coupling with phenol, -OH band has a lower frequency due to the coupling with amino group. However the signal at 1070 cm^{-1} is disappeared indicate the absorption of thione (C=S) form.

A weak absorption band is observed at 2550cm^{-1} which can be assigned to –SH group[59]. The probable reason may be due to the possibility of H-bonding with the –OH and $–NH_2$ group present as substituents on the phenyl and naphthyl ring[57].

The absorption at 2300cm^{-1} are the characteristic –CN group. The IR spectra confirms the presence of compound (12) by the strong sharp band at 1640cm^{-1} for –N=N- (azo group). The absorption band at 1600 $cm^{-1}$are characteristic C=C str in aromatic nuclei[61]. The presence of band at ~1210, 1370 cm-1 in the IR spectrum of (12) (as absent in the IR spectrum of 11) Further confirms the presence of strong C-O str band in phenol of diazotized coupled compound[57]. The absorption at 840 cm^{-1} recognized for the disubstituted benzene[61].

In case of coupling with β-naphthol, absorption at δ2570 cm^{-1} are chacteristics –SH group[59]. On comparing the IR spectrum of (13) with that of (12), the shifting of peaks towards lower frequency at 1675cm^{-1} for the –N=N- group indicating the conjugation of benzene ring[57]. The absorption band at ~1350,1060cm^{-1} are the characteristic C-O str in alcoholic group *(Fig. 9)*.

Coupling with N, N- dimethylaniline absorption at 960 cm^{-1} are characteristic –CH def. group in methyl (Fig. 1.7). the δ value was shifted towards lower frequency 15-20 cm^{-1} in group –N=N- as compared in IR spectrum *(Fig. 10)*.

^{1}H-NMR spectra: In case of coupling with phenol compound, the presence of aromatic proton in the region δ8.4-7.09 gave multiplet. The δ value shifted towards downfield due to the presence of –OH group in the phenyl ring[57]. ^{1}HNMR spectra confirms the compound (12) absorb at δ9.6 for the presence of –OH group. The signals for –SH proton appears as singlet at δ 6.6 ppm.

In case of coupling with β-naphthol, the ^{1}HNMR spectra exhibit multiplet for aromatic protons in the region 7.29-7.20.

Coupling with N,N-dimethylaniline, ^{1}HNMR spectra confirms the compound (14) by the signal observed at δ2.97 ppm indicates for the presence of $–CH_3$ group. The nine aromatic protons give multiplet in the range between 7.8-6.9.

UV ABSORPTION STUDIES OF SYNTHESISED TRIAZOLE AND DIAZOTISED COMPOUNDS

(A) 5-phenyl-4-amino-3-mercapto-1,2,4-triazole.

UV spectra of compound (11) has been recorded in different solvents in the region 400-200nm and the observed characteristics absorption data are given in Table 1 and 2.

The absorption spectra of the investigated triazoles were recorded in different organic solvent of different polarity at room temperature. Triazoles exhibits an intense absorption band in the near ultra-violet

region, perhaps due to the presence of C=C group which gives a π-π^* band at longer wavelength than either C=N or N=N[62]. In table 1.1 absorption data indicates that UV (EtOH) λ_{max} = 242 nm. However, in Table 2 absorption data indicates for (11) UV (DMF) λ_{max} = 255 nm. The interpretation of this UV absorption may be based upon solute-solvent H-bonding. The n- π^* transitions is predominantly observed in aprotic solvent like DMF, because of increase in polarity of the excited state of the solvent [61-63]. The increasing in H-bonding of the DMF with the compound results a bathochromic shift. However, ethanolic solution of triazole shows blue shift on irradiation**.**

On comparing the UV spectrum of (13) with that of (11), the shifting of towards higher wavelength at 450 nm of compound (13) due to the presence of the auxochromic group which shows bathochromic shift in the compound *(Figure 5)***.**

(B) Dye of 5-phenyl-4-amino-3-mercapto-1,2,4-triazole with phenol

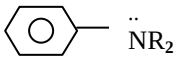
UV spectra of compound (12) have been recorded in different solvent in the region 600-400 nm and the observed characteristic absorption data are given in Table 3.

The absorption spectra of (12) indicate the red shift (460 nm) due to +R (electron donating group) effect of the auxochromic group *(Figure 4).*

(C) Dye of 5-phenyl-4-amino-3-mercapto-1,2,4-triazole with N, N-dimethylaniline

Spectra of (14) have been recorded in different solvents in the region 600-400 nm and the observed characteristic absorption data are given in Table 4.

The absorption maxima of compound (14) is observed at 460nm. Shifting of λ towards the higher wavelength due to the conjugation in the structure of



Thus it will leads to the bathochromic shift.

Antiviral Activity against animal virus:

5-phenyl-4-amino-3-mercapto-1,2,4-triazole (11) and their diazotized compounds (12 to 14) were screened for their antiviral activity against two animal viruses viz. Japanese encephalitis virus (JEV) (P20778) and Herpes Simplex virus type I (HSV-I) (753166) *in vitro* and *in vivo*.

Maintenance of Viruses:

Japanese Encephalitis Virus (JEV)

It was maintained by intra-cerebral passage in 1-3 days old suckling albino Swiss mice. The brains of the infected mice with specific paralytic symptoms were triturated and 10% homogenate (w/v) was made in phosphate buffered saline (PBS) pH 7.2. The mean lethal dose (LD_{50}) of the virus in mice was calculated before each experiment[64].

Herpes Simplex Virus Type –I (HSV-I)

Virus was maintained in 15-16g of albino Swiss mice by the same route as JEV and 10% virus homogenate (w/v) was prepared and LD_{50} calculated as for JEV.

Maintenance of Cells:

Vero cells were maintained in minimum essential medium (MEM) (Sigma, USA) with

10% foetal bovine serum (Gibco, USA) and 100 units of gentamycin/ml were added.

Cytotoxicty Test and Antiviral Assay *in Vitro*

Cytotoxicity and antiviral assay of the compounds were performed by standard method[65]. Confluent monolayer of vero cells were loaded with two fold serial dilutions of the compound. The treated cultures were incubated for a period of 24h at 37^0C. After incubation the plates were observed microscopically for the evidence of cytotoxicity such as distortion, swelling and sloughing of cells. For antiviral assay the virus was allowed to adsorb onto cell monolayer for a period of 90 min at 37^0C. The unadsorbed virus was removed by decanting of plates and 0.1ml of MEM containing 2.5% foetal bovine serum (FBS) was filled in each cell. Non-toxic dilution 125 to 4µg/ml of the compound was added to the cells. The culture plates were incubated at 37^0C for 72h and examined microscopically for evidence of cytopathogenicity caused by virus and its inhibition with the compounds.

Antiviral Assay *in Vivo:*

Swiss albino mice of 30-35 days old (weighing 15-16g) were randomly bred and maintained in the division laboratory. Animals were used in all the in vivo standard pellet diet (Hindustan Liver Ltd., Bombay) and had access to water *ad libitum.* The animals were maintained at a temperature of 25-30^0C in clean stainless cages. Each experimental group consisted of minimum 6 mice with male and female in the ratio 1:1.

In vivo assay was done according to the method described earlier[66]. Mice of 15-16g body weight were injected with the test compound intraperitoneally once a day. The treatment was started 18h before virus challenge, followed by two consecutive administrations at 24h interval.

After 18 h of the first dose the animals were challenged with 5-10 LD50 of virus subcutaneously. The animals were observed morning and evening for a period of 21 days to record their mortality with specific paralytic symptoms. The average survival time (AST) as well as increase in AST were calculated. The virus infected animals (control) were given PBS in place of drug.

Table 1 Absorption data for 5-phenyl-4-amino-3-mercapto-1, 2,4-triazole (11) in ethanol solvent

λ_{max}(nm)	Absorption
200	0.491
210	0.556
230	1.074
240	2.419
242	2.445
245	2.102
250	1.371
260	0.967
270	0.865
280	0.815

Table 2 Absorption data for 5-phenyl-4-amino-3-mercapto-1,2,4-triazole (11) in DMF solvent

λ_{max} (nm)	Absorption
200	0.243
210	0.278
220	0.564
230	0.602
240	0.845
250	0.963
255	0.954
256	0.903
260	0.699
265	0.246
270	0.140
280	0.120

Table 3 Absorption data of compound (12) in different solvents

λ_{max}(nm)	Alco.	Acetonitrile	DMF
410	0.950	0.961	0.980
420	0.963	0.979	0.998
430	1.056	1.055	1.089
440	1.022	1.078	1.094
450	1.034	1.057	1.048
460	0.979	0.998	1.036
470	0.909	0.987	0.994
480	0.688	0.824	0.902
490	0.504	0.702	0.846
500	0.333	0.546	0.653

Table 4 Absorption data of compound (13) in different solvents

λ_{max}(nm)	Alco.	Acetonitrile	DMF
410	0.00	0.702	0.815
420	0.82	0.822	0.842
430	0.86	0.802	0.937
440	0.90	0.918	1.189
450	0.93	0.975	1.081
460	0.85	0.822	0.983
470	0.84	0.826	0.928
480	0.83	0.818	0.869
490	0.57	0.625	0.663
500	0.41	0.523	0.559
510	0.28	0.361	0.408
520	0.16	0.199	0.217

Table 5 Absorption data of compound (14) in different solvents

λ_{max}(nm)	Alco.	Acetonitrile	DMF
410	0.540	0.533	0.560
420	0.630	0.585	0.642
430	0.705	0.720	0.765
440	0.811	0.796	0.838
450	0.830	0.844	0.972
460	0.859	0.880	0.994
470	0.750	0.832	0.901
480	0.413	0.550	0.623
490	0.201	0.321	0.389
500	0.108	0.206	0.252

Table 6 Cytotoxicity test of 1,2,4-triazole and their diazotized derivatives.

S. No.	Name of Compound	Concentration of compound (ppm)	Microscopic observations
1	11	100	Swelling Observed
		500	No Swelling
		1000	No Swelling
2	12	100	Sloughing observed
		500	Small swelling
		1000	No Swelling
3	13	100	Swelling Observed
		500	No Swelling
		1000	No Swelling
4	14	100	Sloughing observed
		500	Small swelling
		1000	No Swelling

Table 7 Cytopathogencity test of 1,2,4-triazole and their diazotized derivatives.

S. No.	Name of Compound	Conc. of compound (ppm)	Microscopic observations
1	11	100	Minute Swelling observed
		500	No swelling
		1000	No swelling
2	12	100	Minute sloughing observed
		500	Small swelling observed
		1000	No swelling
3	13	100	Small swelling observed
		500	No swelling
		1000	No swelling
4	14	100	Sloughing observed
		500	Small swelling
		1000	No swelling

Table 8 Antiviral test of 1,2,4-triazole and their diazotized derivatives on Albino Mice observed for 21 days.

S. No.	Name of Compound	Conc. of compound (ppm)	Response to Japanese Encephalitis Virus (JEV)
1	11	100 500 1000	Died after 20th day Developed paralytic symptoms Alive and active
2	12	100 500 1000	Developed paralytic symptoms Alive and active Alive and active
3	13	100 500 1000	Died after 20th day Developed paralytic symptoms Alive and active
4	14	100 500 1000	Developed paralytic symptoms Alive and active Alive and active

Table 9 Antiviral test of 1,2,4-triazole and their diazotized derivatives on Albino Mice observed for 21 days.

S. No.	Name of Compound	Conc. of compound (ppm)	Response to Herpes Simplex Virus Type–I (HSV-I)
1	11	100 500 1000	Died after 21st day Developed paralytic symptoms Alive and active
2	12	100 500 1000	Developed paralytic symptoms Become lazy Alive and active
3	13	100 500 1000	Developed some paralytic symptoms Developed paralytic symptoms Alive and active
4	14	100 500 1000	Developed paralytic symptoms Become lazy Alive and active

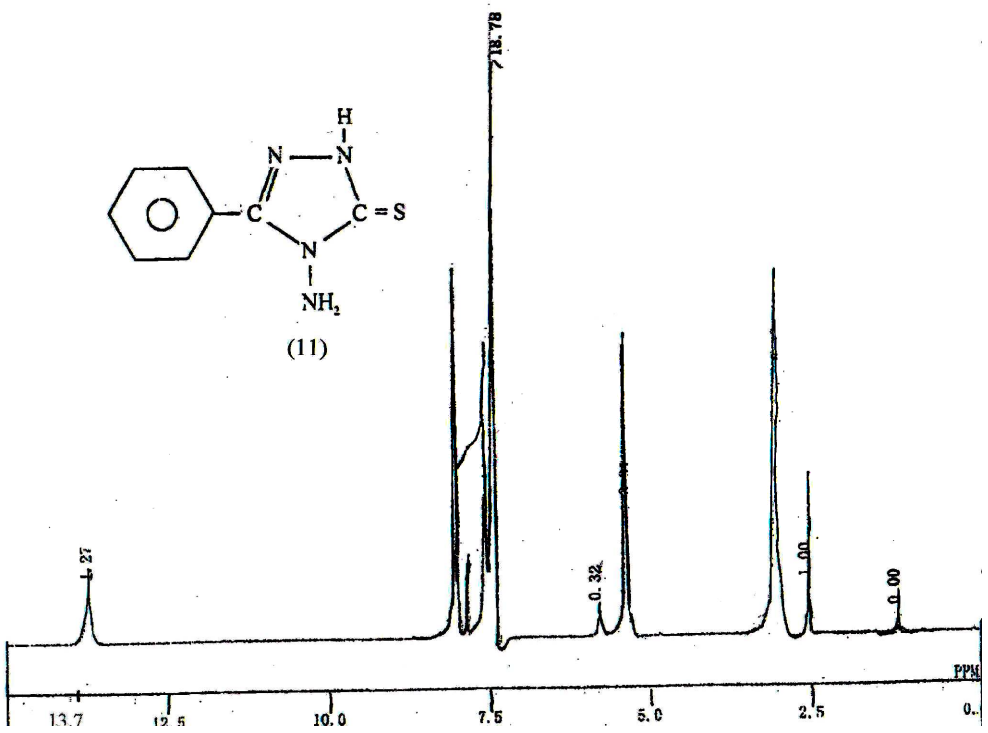


Figure 7. IR spectra of 5-phenyl-4-amino-3-mercapto-1,2,4-triazole (11).

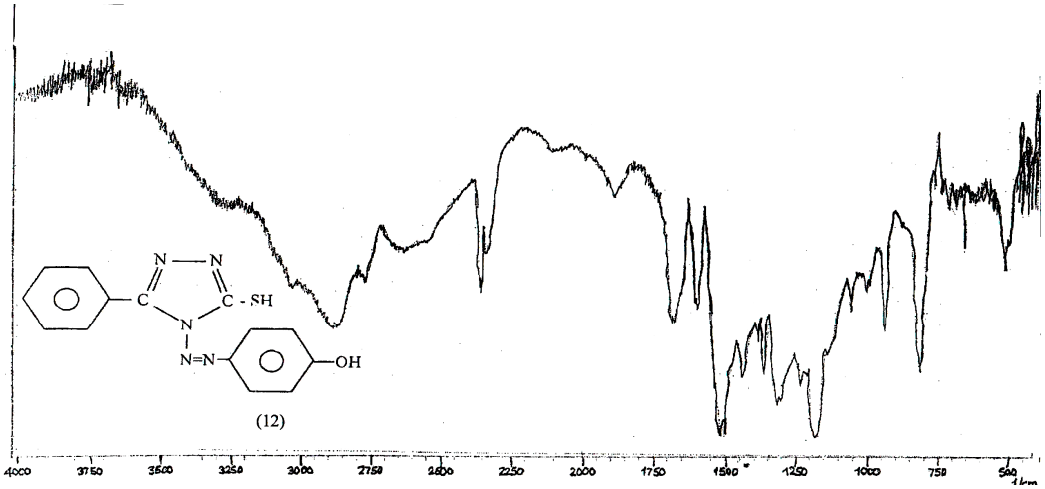


Figure 8. IR spectra of phenole diazotized compound (12).

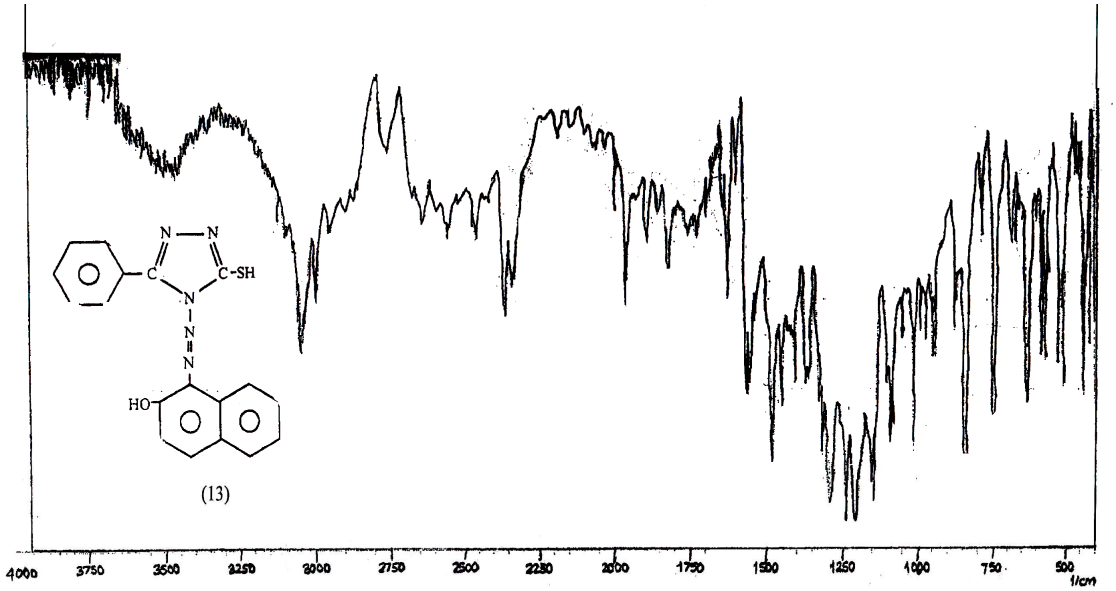


Figure 9. IR spectra of β-naphthol diazotized compound (13).

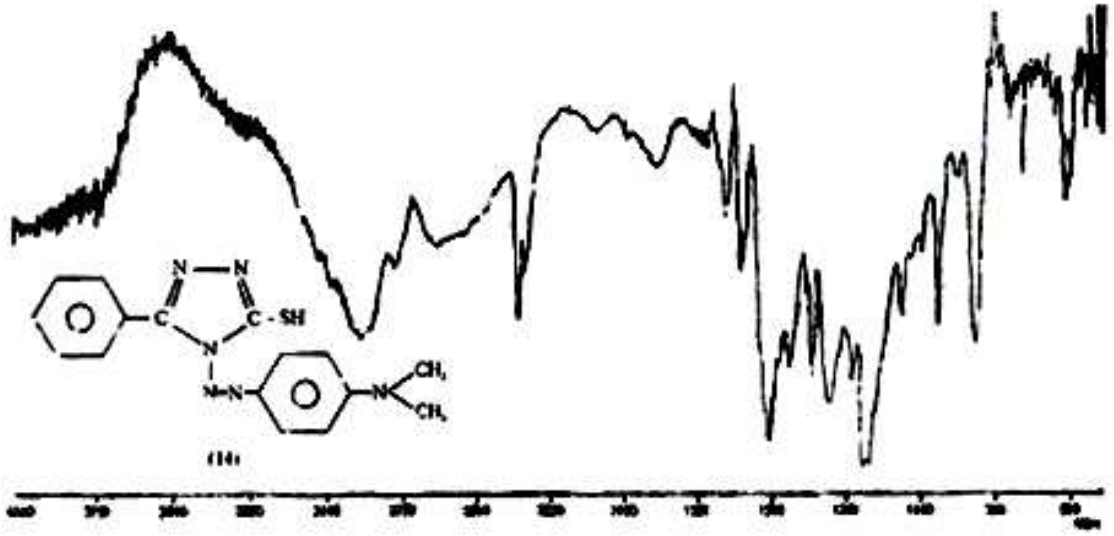


Figure 10. IR spectra of N, N-dimethyl aniline diazotized compound (14).

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